

Canine vector borne diseases of zoonotic concern in three dog shelters in Peninsular Malaysia: The importance of preventive measures

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Received 9 July 2016; received in revised form 10 November 2016; accepted 13 November 2016

Abstract. This study investigated canine vector borne diseases (CVBDs) of zoonotic significance in three major shelters in Peninsular Malaysia in order to highlight the importance of shelter management in diseases prevention. Since crowded housing has the potential to bring vectors and infected dogs into close proximity, the prevalence of CVBDs infection is always higher in sheltered dogs in comparison to pet dogs. Serum was collected from 90 adult dogs (30 from each shelter) from three shelters in different regions of Peninsular Malaysia (Shelter A, B and C). The prevalence of *Dirofilaria immitis*, *Ehrlichia canis*, *E. ewingii*, *Anaplasma phagocytophilum*, *A. platys* and *Borrelia burgdorferi* in dogs residing in three shelters with different management protocols was investigated by using rapid clinical test kits, SNAP® 4Dx® Plus (IDEXX Laboratories, Westbrook, ME). The most common pathogen detected was *Ehrlichia* spp. (55.6%, 50 out of 90 dogs), followed by *Anaplasma* spp. (16.7%; 15 out of 90 dogs). *Dirofilaria immitis* could only be detected in one of the shelters (10.0%; nine out of 90 dogs). No evidence of *B. burgdorferi* was detected in all three shelters. Forty one out of 90 dogs (45.6%) were infected with at least one pathogen meanwhile 14 out of 90 dogs (15.6%) were infected with more than one pathogen. It is of crucial importance to practice preventive measures during the admission of the new animals into the facilities. Occasional treatment of the dogs (as practiced in Shelter B) was not effective when compared to scheduled preventive measures. Future studies should emphasize the isolation and additional molecular characterization of vector borne pathogens in Peninsular Malaysia. In addition, studies that define the tick species that infest dogs in this region would help to elucidate their role as vectors for disease transmission.

INTRODUCTION

Canine vector borne diseases (CVBDs) are not only of major concern in animal health, but of major public health importance, especially in tropical countries. These diseases were caused by varying number of arthropod vectors, infectious agents and hosts. Their epidemiology is greatly differed from one geographic zone to another and from time to time. With the

increasing proximity of human and animal populations, no doubt the risk of exposure of animals and humans to the same vector borne pathogens has increased notably (Vorou *et al.*, 2007; Otranto *et al.*, 2009; Rani *et al.*, 2010; Chomel, 2011). Dogs, as one of the closest companion to human being, serve as a competent reservoir host of several zoonotic agents, such as *Hepatozoon canis*, *Ehrlichia canis*, *Anaplasma platys*, *A. phagocytophilum*,

Bartonella henselae, *Borrelia burgdorferi*, *Dirofilaria repens* and *D. immitis* (Otranto *et al.*, 2009). Depending upon the presence and abundance of arthropod vectors, dogs can be infected simultaneously with a large number of vector borne pathogens. The ability of some of these pathogens to cross the species barrier raises the growing medical interest in CVBDs, which is directly related to animal welfare and public health (Nicholson *et al.*, 2010).

Malaysia is situated at the equatorial region and its climate has relatively high levels of temperature (mean 26.5°C, range from 22 to 33°C) and humidity (range from 80% to 90%; Mekhilef *et al.*, 2012). The combination of the tropical climate and abundance sources of water supports a variety of arthropod vectors of zoonotic CVBDs, including mosquitoes and brown dog tick (*Rhipicephalus sanguineus*). These vectors pose an increased risk of diseases transmission among the intensively housed dogs, including sheltered dogs. On the other hands, sheltered dogs are commonly relinquished as unwanted dogs by their owner or collected from free roaming strays. It is unlikely that these dogs were routinely provided with preventive medicine against the CVBDs in the past.

For developing countries, vector borne diseases which related with high mortality in humans, such as dengue, constitute a high-priority issue for public health. As a result, other CVBDs remain a considerably lower priority, despite the high number of stray dogs, high-density of vector populations and high risk of zoonotic transmission by arthropod vectors living in the same microenvironments. In sheltered dogs, CVBDs receive minimum attention and data on the distribution of arthropods and CVBDs are scant and anecdotal due to the lack of surveillance at regional levels. The recent publications regarding the CVBDs in Malaysia reported 13.3% prevalence for *A. platys* in dogs that were surrendered to an animal shelter (Mokhtar *et al.*, 2013). *Ehrlichia canis* had also been reported with prevalence

as low as 2.0% in 500 pet and street dog populations in Peninsular Malaysia (Nazari *et al.*, 2013). Information on prevalence of CVBDs in different shelters with different management protocol in Malaysia remains scarce.

In order to investigate in depth the impacts of shelter management protocols on prevalence of CVBDs, this study was performed to compare the prevalence of the CVBDs in three different dog shelters with different management by using SNAP® 4Dx® Plus (IDEXX Laboratories, Westbrook, ME), which is a rapid, highly sensitive, and specific test that can diagnose more than one pathogens (*Dirofilaria immitis* antigen, and *E. canis*, *E. ewingii*, *A. phagocytophilum*, *A. platys* and *B. burgdorferi* antibodies), including co-infections.

MATERIALS AND METHODS

Animals and samples collection

Between January and March 2015, blood was collected from three major dog shelters located in different states within Peninsular Malaysia. All these three shelters were run by different private animal welfare organizations and practice no euthanasia policy. Demographic and shelter management information was collected from each shelter and medical history of individual dog was not available. This study was carried out in accordance with the guidelines of the Animal Care and Use Committee (ACUC), Universiti Putra Malaysia (UPM/IACUC/AUP-080-FPV-SYP-2013).

Serological testing

Approximately 3ml of blood was collected from the sheltered dogs via cephalic venipuncture and serum was separated by centrifugation and stored at -80°C until further analysis. Commercially available in-clinic rapid test kit (SNAP® 4Dx® Plus, IDEXX Laboratories, Westbrook, ME) was used for the simultaneous detection of antibodies against *E. canis*, *E. ewingii*, *A. phagocytophilum*, *A. platys* and *B.*

burgdorferi and *D. immitis* antigen in serum obtained from dogs. Frozen serum samples were thaw at the room temperature 18–25°C for 30 minutes, three drops of test sample were mixed with four drops of conjugate and applied to the flow matrix, as described in the manufacturer’s instructions. Within 30-60 seconds, the sample flowed across the result window and reached the activation circle. Once color first appeared in the activation circle, the activator was pushed firmly and the result was read after eight minutes by using IDEXX SNAP® ShotDx® Analyzer (IDEXX Laboratories, Westbrook, ME).

Data analysis

Test results collected were compiled accordingly and prevalence of *D. immitis*, *E. canis*, *E. ewingii*, *A. phagocytophilum*, *A. platys* and *B. burgdorferi* were calculated and expressed in percentage. The seroprevalence was then compared among each dog shelter.

RESULTS

Characteristic of dogs, dog shelters and seroprevalence of CVBDs infections in each shelter

Throughout the study, 90 dog serum samples were collected from three animal shelters (30 per each shelter; Table 1). There was slightly higher proportion of male dog (53.3%, 48 out of 90 dogs) in the shelters in comparison to female dogs (46.7%; 42 out of 90 dogs). All the dogs were local mixed breed from adult age. During the physical examination, dogs were clinically healthy, except some of the dogs from shelter A showed pale mucous membrane. Generally,

41 out of 90 dogs (45.6%) were infected with at least one pathogen meanwhile 14 out of 90 dogs (15.6%) were infected with more than one pathogen. The most common pathogen detected was *Ehrlichia* spp. (55.6%, 50 out of 90 dogs), following by *Anaplasma* spp. (16.7%; 15 out of 90 dogs). *Dirofilaria immitis* could only be detected in one of the shelters (10.0%; nine out of 90 dogs). No evidence of *B. burgdorferi* was detected in all three shelters.

Shelter A

This particular shelter was located in Pahang, due to the severe shortage in funding, practiced no preventive medicine in the shelter and home remedy treatment was given once dogs found sick. The shelter was located at the rural area and surrounded by woods, pond and river (Figure 1a). With the population of nearly 300 dogs, they were kept freely in the shelter compound together with other species of animals including cats, goats, cattle, and chicken. In general, dogs were severely infected by ticks and some of the dogs were anemic and showed pale mucous membrane. Twenty one out of 30 dogs (70.0%) showed positive for *Ehrlichia* spp. and nine out of 30 dogs (30.0%) from this shelter showed positive for *D. immitis*. Only five out of 30 dogs (16.7%) were positive for *Anaplasma* spp. Mix infections were found in five out of 30 dogs (16.7%), where *D. immitis* and *Ehrlichia* spp. were found. None of the dogs were found positive for *B. burgdorferi*.

Shelter B

Shelter B was located in Selangor. Dogs in shelter B were confined in different groups in the building with concrete floor. Dogs took turn to run freely in pens that have grasses

Table 1. Prevalence of the various canine vector borne diseases in sheltered dogs in Peninsular Malaysia

Shelter	n	<i>Dirofilaria immitis</i>	<i>Ehrlichia</i> spp.	<i>Anaplasma</i> spp.	<i>Borrelia burgdorferi</i>	One pathogen	More than one pathogens
A	30	9 (30.0%)	21 (70.0%)	5 (16.7%)	0 (0.0%)	25 (83.3%)	5 (16.7%)
B	30	0 (0.0%)	22 (73.3%)	9 (30.0%)	0 (0.0%)	15 (50.0%)	8 (26.7%)
C	30	0 (0.0%)	7 (23.3%)	1 (3.3%)	0 (0.0%)	6 (20.0%)	1 (3.3%)



Figure 1 (a). Shelter A was located at the rural area and surrounded by woods, pond and river. (b) Dogs in shelter B were confined in different groups in the building with concrete floor and they took turn to run freely in pens that have grasses. (c) Dogs in shelter C were confined in the concrete area and freely mixed with each other.

(Figure 1b) and also pond. In this particular shelter, amitraz was sprayed on the dog's house weekly and there was no regular dipping bath practiced on the dogs. Topical treatment with 0.29% w/w fipronil spray (Frontline® spray, Merial Animal Health, Athens, GA) was applied on the dog whenever there were ticks found on the body. Frontline is against ticks and fleas and not against mosquitos. Doxycycline (10mg/kg, once a day orally) was given to the dogs once they showed evidence of loss of appetite and fever. During the blood sampling, dogs were healthy and no ticks found on the body. Despite of the environmental preventive measures, it has the highest seroprevalence of the *Ehrlichia* spp. (73.3%, 22 out of 30 dogs) and 30.0% (nine out of 30 dogs) of the dogs were seroprevalence positive for *Anaplasma* spp., respectively. Eight out of 30 dogs (26.7%) were positive for mix infections with combination of *Ehrlichia* spp. and *Anaplasma* spp. There was no evidence of *D. immitis* and *B. burgdorferi* was found in this shelter.

Shelter C

Dogs in shelter C which located in Johor, were confined in the concrete area and freely mixed with each other (Figure 1c). In shelter C, they practice strict policies regarding the admission of the animals. During the first introduction of the animals into the shelter, dipping bath would be applied and doxycycline treatment (10mg/kg, once a day orally) was given during the first month

introduction into the shelter as prophylaxis treatment. Dipping bath by using flumethrin 1% pour-on (Bayticol, Bayer AH, Germany) was performed every three weeks on the dogs. Only seven out of 30 dogs (23.3%) were positive to *Ehrlichia* spp., and one out of 30 dogs (3.3%) was positive for *Anaplasma* spp. The only dog that was tested positive for *Anaplasma* spp. was infected by *Ehrlichia* spp. concurrently. In this dog shelter, there was no evidence of *D. immitis* and *B. burgdorferi*.

DISCUSSION

The paper describes prevalence of CVBDs in a cohort of sheltered dogs, as well as detailing the different shelter management protocol. Dog population in Peninsular Malaysia can be divided into pets, working dogs and stray dogs. In developing countries, priority for controlling stray animal population includes an effective, sustainable and practical approach, which has always been an issue due to the limitation in financial support and lack of public awareness. Stray dogs remain a serious public health problem. In addition to carrying serious diseases, these dogs are frequently infested with large numbers of ticks, potentially serving as reservoirs. In order to help and accommodate this population, there is drastically increased number of the animal shelters. With the overpopulation of the stray animals, animal shelters are normally operating with

insufficient funding, staff, and expertise in managing the disease. Each and every dog shelter has their own management protocol for the CVBDs depending of the socio-economic status and manpower (Tzipory *et al.*, 2010).

Dirofilaria immitis was identified in only one shelter (30.0%; nine out of 30 dogs), which is located in the rural area. The zero incidence of heartworm observed in Shelter B and C was probably due to a number of reasons. Shelter B and C were located in urban area where the dogs were confined in the concrete building, in an environment with lower presence of mosquitoes. With proper mosquito control measures the number of the mosquitoes in the residential area, might be reduced. In comparison to the dogs in Shelter B and C, dogs in Shelter A had not been treated regularly with doxycycline. McCall (2011) suggested that doxycycline at 10 mg/kg orally twice a day for 30 days has efficacy against migrating tissue-phase larvae and juvenile worms and will delay or restrict microfilarial production by adult female worms, which gradually reduce the incidence of heartworm disease. Without further investigation, we could not conclude the role of doxycycline in this study. Heartworm disease caused by *D. immitis* can cause serious cardiopulmonary disease in dogs, but not in human. Infected people might remain asymptomatic or not associated with severe clinical symptoms (Theis *et al.*, 2001; Lee *et al.*, 2010). However, due to its zoonotic nature, proper control programs should be planned in order to eliminate this pathogen from the canine population when the existence of the pathogen in dog population is confirmed.

In comparison to Nazari (2013), the prevalence of *Ehrlichia* spp. in this study was relatively high (23.3–73.3%) despite of the molecular screening by polymerase chain reaction (PCR) was performed in the study of Nazari (2013). The most likely explanation is the fact that in the previous study about 60% of the studied population were owned dogs. In comparison to shelter

animals, which were severely neglected, pet dogs had higher chance to get treatment, as well as tick prevention. In addition to that, the studied sheltered populations might have different management and preventive measures. As a country with a tropical climate, *R. sanguineus* is present in Peninsular Malaysia throughout the year and can infest the dogs. It was detected on numerous dogs during this study and was the only tick species that had been detected on infested dogs in Peninsular Malaysia currently. This information should be taken into account during the planning of control program against this tick species. At this time, *E. canis* is the only *Ehrlichia* spp. known to infect dogs in this country. *Ixodes* ticks, which are the vectors of *E. ewingii* have not yet been identified. Hence, we believe that all the seroprevalence that reported in this study belongs to *E. canis* rather than *E. ewingii*. This pathogen had been reported causing approximately 20 human patients admitted to the emergency clinic in Venezuela with clinical signs compatible with human monocytic ehrlichiosis (HME; Perez *et al.*, 2006). Its zoonotic potential should not be underestimated. *Ehrlichia canis* infection should be considered among differential diagnosis for the febrile episodes with unknown origin in this region of the world.

The prevalence of *Anaplasma* spp. in this study was at 16.7%. This bacterium had been reported before in a dog shelter in Peninsular Malaysia (Mokhtar *et al.*, 2013) and other regions of the world (Yabsley *et al.*, 2008). In similarity with *E. ewingii*, *A. phagocytophilum* is transmitted by *Ixodes* ticks (Stuenkel, 2007), which has not yet been reported in Peninsular Malaysia. Therefore, in this study, we assume that the seropositive result was due to *A. platys*. This pathogen generally does not cause severe clinical signs in dogs and it has been found worldwide with higher incidence in tropical and subtropical regions (Chomel, 2011). It is not thought to be zoonotic at the same time (Cohn, 2003).

As one of the major emerging tick borne diseases of humans, *B. burgdorferi* which causes the Lyme disease had not been detected in any of three shelters. The most likely explanation is the insufficient investigation on ticks' distribution in Peninsular Malaysia or this disease might either absence or present in a very low prevalence in this country. This disease has been reported extensively in Europe, especially Germany, Austria and Switzerland (Shapiro, 2008). Nevertheless, *Ixodes* ticks had been reported before in other Southeast Asia countries (Kollars *et al.*, 2001; Parola *et al.*, 2003). Further investigation in the future in a bigger sample size is warranted.

By using this rapid test kit, 45.6% (41 of 90) of the dogs in this study were positive to at least one pathogen. The overall seroprevalence in these sheltered dog populations was surprisingly high and suggests that canine exposure to vector borne pathogens in Peninsular Malaysia were extensive, especially in sheltered dogs. Transmission of multiple pathogens by the same tick vector or chronic infection by different tick vector at different time points can lead to simultaneous infection and animals infected with these agents can experience a wide range of clinical manifestation. In this study, only anemia was noticed in some of the dogs. Concurrent infection with multiple pathogens can lead to a more severe disease presentation, which may help explain the variety of clinical manifestations erroneously attributed to CVBDs throughout the world (Dantas-Torres, 2008; Beugnet & Marie, 2009; Menn *et al.*, 2010). In Peninsular Malaysia, financial constraints and cultural barriers substantially impede effective dog control. Most of the shelters are not able to adhere to heartworm and ticks prevention guidelines due to the cost. Preventive measures as practiced in Shelter C are important in order to control the

prevalence of at least some CVBDs. Occasional treatment on the dogs (as practiced in Shelter B) might not be effective in comparison to scheduled preventive measures. It is of crucial importance to practice preventive measures during the admission of the new animals into the facilities. Nevertheless failure to provide preventive medicine to susceptible dogs housed in close proximity to infected dog in the shelter creates a high risk for transmission of new infection.

CONCLUSION

Future studies should emphasize the isolation and additional molecular characterization of vector borne pathogens in Peninsular Malaysia. Our current study was only focused on three major shelters in different states, which is not enough in order to provide comprehensive information regarding distribution of CVBDs in Peninsular Malaysia. In addition, studies that define the tick species that infest dogs in this region would help to elucidate their role as vectors of disease transmission. It is also of crucial importance to investigate the prevalence of CVBDs in other shelters in different regions as they share the same habitat with human being and its potential threat to human health.

Conflict of interest

The authors declare that they have no competing interests.

Acknowledgements. This study was funded by University Putra Malaysia (Grant no: 9426400). Our profound gratitude to Dr. Goh, who helped link us with the management of the animal shelters. We are most grateful to the animal shelters for willing to participate and providing all the necessary assistance during the course of this research.

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